



## High incidence of antibiotic multi-resistant bacteria in coastal areas dedicated to fish farming

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### ABSTRACT

Marine bacteria exposed to antibiotics in fish farms can acquire antimicrobial resistance by mobile genetic elements and horizontal gene transfer. A total of 872 autochthonous marine bacterial strains was isolated from samples collected from four different fish farms located at northern and southern Italian Adriatic Sea. Resistance to only tetracycline (17%) and to trimethoprim-sulfadiazine (7%) were the most frequent patterns obtained, while flumequine resistance has recorded in only 0.3% of the strains. Comparing strains isolated from coastal areas and fish farms, a significant higher incidence (4% versus 10%) of multi-resistant strains in aquaculture centers was found. Significant differences in antibiotic resistance incidence were also detected among the four fish farms due probably to different approaches in farm management and the more or less frequent use of antibiotics. Antibiotic-resistant and multi-resistant strains isolated constitute an environmental reservoir directly involved in the seafood chain and might represent a public health concern.

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### 1. Introduction

Large-scale marine aquaculture has been associated with environmental and microbiological concerns worldwide as a consequence of intensive culture and high stocking density. The main coastal areas used for extensive and semi-intensive fish farming in Italy are located at the North Adriatic region near the foci of large rivers, such as Isonzo, Tagliamento and Po, and at the South Adriatic region near the Promontorio of Gargano. There are different types of environmental areas used for fish farming in this large coastal region, namely “lagoons” or large brackish areas (Venice, Grado, Caorle, Marano, Varano and Lesina), “sacche” defined as brackish environments of the Delta of Po river with wide bays communicating with the sea through large mouths only partially enclosed by sand banks and “valli” which are sectors of lagoon enclosed by embankments and characterized by brackish water (Landoli, 2000). Italian fish culture production mainly follows two procedures: productive management of the coastal environment (mostly lagoons), performed principally in marine and brackish water using extensive and semi-intensive procedures, and the intensive farming of highly valuable fish and shellfish species, carried out mostly in tanks and cages. In recent years, Italian

aquaculture has been characterized by a strong increase in output due to the technological optimization of existing installations as well as to the application of innovative technology and the setting up of new facilities (Landoli, 2000). However, several hazards, such as infection diseases in fish and shellfish, constitute an important economical concern and reduction or elimination of these pathologies, a real challenge. In addition to the marine autochthonous microbiota, including non-pathogenic species of *Vibrio*, *Aeromonas* and *Photobacterium*, several species, such as *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio anguillarum*, *Photobacterium damsela* and *Aeromonas salmonicida* among others, are known to induce severe bacterial diseases in aquaculture livestock (Jayasree et al., 2006; Balboa et al., 2012; Labella et al., 2010). The use of chemicals and antibiotics for the treatment or prevention of these infections is the approach used in most cases. In Italy, the use of antibiotics amoxicillin, tetracycline and oxytetracycline, flumequine, trimethoprim and the association of trimethoprim with sulfadiazide is allowed by the current legislation. Marine bacteria exposed to antibiotics inside or outside the fish farming environment can acquire antimicrobial resistance transferable by mobile genetic elements and horizontal gene transfer (Serrano, 2005) and can cause changes on the coastal environments. Pollution of coastal areas represents one of the most important environmental problems because it causes economic and tourism damages as well as affects health quality. It has been shown that antibiotics released into the aquatic environment are of great concern for the contamination of water used for drinking, irrigation and recreation, the widespread

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occurrence of bacterial resistance to antibiotics and the negative effect on microbes which play vital role in nutrient cycling and regeneration of nutrients in aquatic ecosystems (Costanzo et al., 2005). In Italy, it has been reported the emergence of resistant bacterial strains as a consequence of the massive use and abuse of some antibiotics as described for chloramphenicol in trout farms (Laganà et al., 2011). High frequency (about 80%) of resistance to ampicillin and other beta-lactams has been reported in microorganisms isolated from fish, seafood and environment in Italy, in contrast to the susceptibility observed for other antibiotics, such as quinolones and trimethoprim–sulfamethoxazole (Ottaviani et al., 2001; Zanetti et al., 2001; Ferrini et al., 2008). Recently, enterococci resistant to tetracycline, ampicillin and erythromycin have been isolated in a coastal area close to an aquaculture center in the central Adriatic Sea (di Cesare et al., 2012) suggesting an involvement of fecal contamination indicators in antibiotic-resistant (AR) bacterial marine reservoirs.

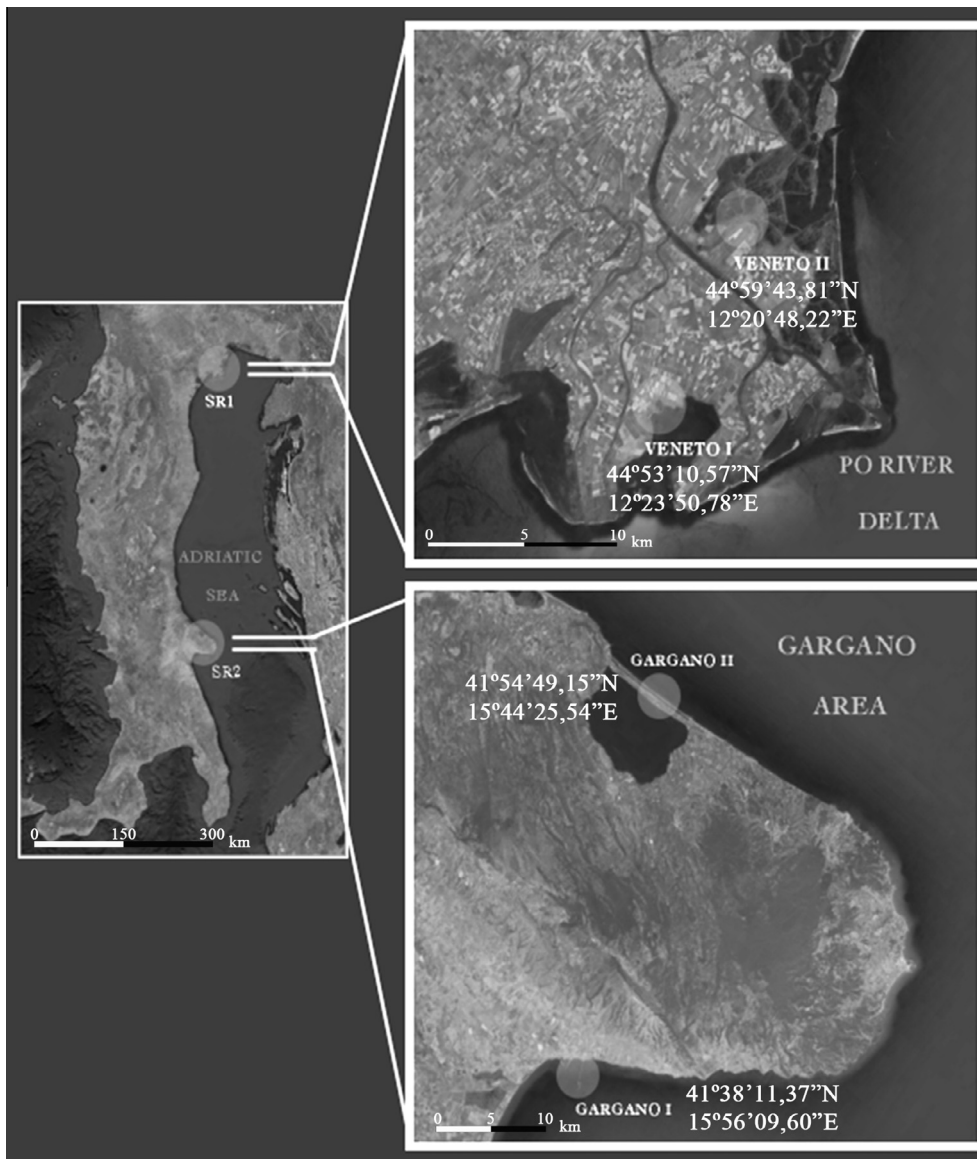
The transfer of multiple resistances to antibiotic from resistant to susceptible bacteria is a major concern in fish and shellfish

farming and for this reason much more research is needed regarding the incidence of multi-resistant bacterial isolates. In this study, we have analyzed in four different aquaculture centers located at northern and southern Adriatic coast, the incidence of resistance to each one of the antibiotics in use and that of multi-resistant bacterial strains showing resistance to at least three of those drugs and have compared it to the AR strain incidence in coastal areas not dedicated to aquaculture.

## 2. Materials and methods

### 2.1. Sampling and processing of aquaculture samples

Geographical areas and sampling sites from which samples were collected are indicated in Figs. 1 and 2. A series of aquaculture samples was collected, during the year 2011, in four different fish farms located along the Adriatic Sea between the Venetian Lagoon and the Gargano area in Puglia (Italy) and in a coastal site in the



**Fig. 1.** Sampling geographical areas of the Adriatic Sea region from Italy coast. SR1: sampling region 1, corresponds to Po river delta (Veneto region, North Italy) where two aquaculture centers named as Veneto I and Veneto II were studied. SR2: sampling region 2, corresponds to Gargano area (Puglia region, South Italy) where were situated the Gargano I and Gargano II aquaculture centers.

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